

Attachment to Amendment and Reply dated March 7, 2001

Marked-up Claims 4-5, 7-12, 14-25 and 27-36

4. (Amended) A method [according to claim 3] for categorizing nucleic acid, wherein said method comprises:

(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein said endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has a sticky end of a known base sequence and of a known common length extending from a terminal of its double-stranded portion, and wherein each nucleic acid in the nucleic acid population has a double-stranded portion;

(ii) [wherein prior to contacting the nucleic acid population with the oligonucleotide sequences,] contacting the nucleic acid population [is contacted] with an adaptor to ligate the adaptor to a terminal of each nucleic acid in the nucleic acid population, wherein said adaptor comprises a double-stranded primer portion having a known base sequence, and a single-stranded portion complementary to the known sticky end of the nucleic acids in the nucleic acid population;

(iii) contacting the nucleic acid population with one or more oligonucleotide sets; and

(iv) categorizing the nucleic acid by isolating nucleic acid which correctly hybridizes to an oligonucleotide set, wherein each oligonucleotide sequence in each oligonucleotide set has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the

recognition sequence, the recognition sequence being situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease, wherein each oligonucleotide set has a different recognition sequence.

5. (Amended) [A] The method according to claim 4, wherein each oligonucleotide sequence comprises a first sequence, a second sequence attached to the first sequence and a third sequence attached to the second sequence, in which the first sequence is complementary to the sequence of the primer portion of the adapter, the second sequence is complementary to the known sticky end of the nucleic acids in the nucleic acid population, and the third sequence comprises the pre-determined recognition sequence.

7. (Amended) A method [according to claim 6,] for categorizing nucleic acid, wherein said method comprises:

(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein said endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has a sticky end of a known common length extending from a terminal of its double-stranded portion, wherein said sticky ends have a plurality of different base sequences, and wherein each nucleic acid in the nucleic acid population has a double-stranded portion;

(ii) [wherein prior to contacting the nucleic acid population with the oligonucleotide sequences,] contacting the nucleic acid population [is contacted] with an array of adaptors to ligate an adaptor to a terminal of the nucleic acids in the nucleic acid

population, wherein each adaptor comprises a double-stranded primer portion having a known base sequence, and a single-stranded portion of the same length as the sticky ends of the nucleic acids in the nucleic acid population, all of the possible base sequence of the single-stranded portion of the adaptor being represented in the array of adaptors;

(iii) contacting the nucleic acid population with one or more oligonucleotide set;
and

(iv) categorizing the nucleic acid by isolating nucleic acid which correctly hybridizes to an oligonucleotide set, wherein each oligonucleotide sequence in each oligonucleotide set has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence, the recognition sequence being situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease, wherein each oligonucleotide set has a different recognition sequence.

8. (Amended) [A] The method according to claim 7, wherein each oligonucleotide sequence comprises a first sequence, a second sequence attached to the first sequence and a third sequence attached to the second sequence, in which the first sequence is complementary to the sequence of the primer portion of the adaptors, the second sequence is of the same length as the sticky ends of the nucleic acids in the nucleic acid population, and the third sequence comprises the pre-determined recognition sequence, and wherein in any one group of oligonucleotides having the same recognition sequence all of the possible base sequences of the second sequences are represented.

9. (Amended) [A] The method according to claim 5, wherein the recognition sequence consists of one base.

10. (Amended) [A] The method according to claim 5 [8], wherein the recognition sequence consists of two or more bases.

11. (Amended) [A] The method according to claim 5, wherein in any one group of oligonucleotides having the same recognition sequence the third sequence consists of the recognition sequence and a pre-determined number of bases situated between the second sequence and the recognition sequence, all possible sequences of the pre-determined number of bases in the third sequence being represented in that group of oligonucleotides.

12. (Amended) [A] The method according to claim [1] 4, wherein the nucleic acid population is amplified by PCR prior to reaction with the oligonucleotide sequences.

14. (Amended) A method [according to claim 13,] for categorizing nucleic acid, wherein said method comprises:

(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein said endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has a sticky end of a known base sequence and of a known common length extending from a terminal of its double-stranded portion, and wherein each nucleic acid in the nucleic acid population has a double-stranded portion;

(ii) [wherein prior to contacting the nucleic acid population with the

oligonucleotide sequences,] contacting the nucleic acid population [is contacted] with an adaptor to ligate the adaptor to a terminal of each nucleic acid in the nucleic acid population, wherein said adaptor comprises a double-stranded primer portion having a known base sequence, and a single-stranded portion complementary to the known sticky end of the nucleic acids in the nucleic acid population;

(iii) categorizing the nucleic acid by isolating a nucleic acid wherein both termini of the double-stranded portion of said nucleic acid correctly hybridize to an oligonucleotide sequence by [wherein] contacting a first set of oligonucleotide sequences [is contacted] with the nucleic acid population [in a first step] by:

(a) denaturing the nucleic acid population in the presence of the first set of oligonucleotide sequences to produce a single-stranded nucleic acid population and allowing the single-stranded nucleic acid to hybridise to the first set of oligonucleotide sequences, wherein each oligonucleotide sequence in said first set of oligonucleotide sequences has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence, the recognition sequence being situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease:

(b) immobilizing those nucleic acids which correctly hybridise to the first sequences[,];

(c) extending the correctly hybridised oligonucleotide sequences along the single-stranded portion of the immobilised nucleic acid to form double-stranded nucleic acid[,];

(d) denaturing the double-stranded nucleic acid and removing non-immobilised species to isolate the resulting immobilised single-stranded nucleic acid[,];

(e) contacting the immobilised single-stranded nucleic acid with a second set of oligonucleotide sequences, wherein each oligonucleotide sequence in said second set of oligonucleotide sequences has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence, the recognition sequence being situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease [in a second step,];

(f) extending the correctly hybridised oligonucleotide sequences along the immobilised single-stranded nucleic acid to form double-stranded nucleic acid[,];

(g) denaturing the double-stranded nucleic acid; and

(h) isolating the resulting non-immobilised single-stranded nucleic acid.

15. (Amended) [A] The method according to claim 14, wherein the extended and isolated products of the first step and/or the extended and isolated products of the second step are amplified by PCR.

16. (Amended) [A] The method according to claim 14, wherein the correctly hybridised nucleic acids are immobilised by immobilising the oligonucleotide sequences.

17. (Amended) [A] The method according to claim 16, wherein each oligonucleotide in the first set of sequences carries a biotin residue such that prior to or after hybridising to the nucleic acid the sequence is captured on an avidinated solid phase.

18. (Amended) [A] The method according to claim 16, wherein each oligonucleotide in the first set of sequences is covalently attached to a solid support prior to contacting with the nucleic acid population.

19. (Amended) [A] The method according to claim 14, wherein the recognition sequence of the first and second set of oligonucleotide sequences consists of one base and, prior to performing the first step, the nucleic acid population is sub-divided into 16 wells, each well containing oligonucleotides from the first set of sequences having one of the four possible recognition sequences and wherein in the second step oligonucleotides from the second set of sequences are added to each well, such that all possible combinations of the identities of the first and second set of oligonucleotide sequences and their order of addition to the well are represented in the 16 wells.

20. (Amended) [A] The method according to claim 14, wherein the recognition sequence of the first and second set of oligonucleotide sequences consists of two bases and,

prior to performing the first step, the nucleic acid population is sub-divided into 256 wells, each well containing oligonucleotides from the first set of sequences having one of the 16 possible recognition sequences, and wherein in the second reaction oligonucleotides from the second set of sequences are added to each well, such that all possible combinations of the identity of the first and second set of oligonucleotide sequences and their order of addition to the well are represented in the 256 wells.

21. (Amended) [A] The method according to claim 19, wherein the contents of each pair of wells to which the same pair of oligonucleotide sequences were added but in a different order, are combined to give 10 different wells.

22. (Amended) [A] The method according to claim 20, wherein the contents of each pair of wells to which the same pair of oligonucleotide sequences were added but in a different order, are combined to give 136 different wells.

23. (Amended) [A] The method according claim [1] 4, wherein the oligonucleotide sequences have equalised melting temperatures.

24. (Amended) [A] The method according to claim 23, wherein the melting temperatures are equalised by incorporating one or more analogues of natural nucleotides into the oligonucleotide sequences, the analogues comprising base modifications, sugar modifications and/or backbone modifications.

25. (Amended) [A] The method according to claim [1] 4, wherein the endonuclease is selected such that it cuts the nucleic acid at a site within the recognition site of the endonuclease.

27. (Amended) [A] The kit according to claim 26, wherein the recognition sequence consists of one base.

28. (Amended) [A] The kit according to claim 26, wherein in the recognition sequence consists of two or more bases.

29. (Amended) [A] The kit according to claim 26, wherein in any one group of oligonucleotides having the same recognition sequence, the third sequence consists of the recognition sequence and a pre-determined number of bases situated between the second sequence and the recognition sequence, all of the possible sequences of the pre-determined number of bases in the third sequence being represented in that group of oligonucleotides.

30. (Amended) [A] The kit according to claim 26, comprising two sets of oligonucleotide sequences, each of the oligonucleotides in one set being biotinylated.

31. (Amended) [A] The kit according to claim 26, comprising two sets of oligonucleotide sequences, each of the oligonucleotides in one set being covalently attached to a solid support.

32. (Amended) [A] The kit according to claim 26, additionally comprising an endonuclease.

33. (Amended) [A] The kit according to claim 32, wherein the endonuclease is selected such that when it is reacted with double-stranded nucleic acid, nucleic acids are produced each of which comprises a double-stranded portion.

34. (Amended) [A] The kit according to claim 33, wherein the endonuclease is selected such that the nucleic acids produced have a sticky end of a known common length extending from a terminal of the double-stranded portion, and wherein each sticky end of each nucleic acid in the nucleic acid population has the same known base sequence.

35. (Amended) [A] The kit according to claim 33, wherein the endonuclease is selected such that the nucleic acids produced have a sticky end of a known common length extending from a terminal of the double-stranded portion, and wherein the sticky ends of the nucleic acids in the nucleic acid population exhibit a plurality of different base sequences.

36. (Amended) [A] The kit according to claim 26, wherein the endonuclease is selected such that it cuts the nucleic acid at a site within the recognition site of the endonuclease.